Gas-Liquid Chromatography in the Analysis of Mercury(II) Compounds

by Paul Mushak*

Introduction

Gas–liquid chromatography (GLC) in recent years has found application in the analysis of various metals, either singly (*1*) or in tandem function with mass spectrometry (*2*), and presently is gaining wide acceptance in the analysis of organomercury(II) compounds. Since increasing use of GLC in mercury analysis may be anticipated for both research and monitoring purposes, it is of value at this point in time to review critically the status of GLC analysis of mercury compounds, including the relevant chemistry and biocoordination chemistry of mercury in its various chemical forms.

General Consideration of Gas-Liquid Chromatography of Mercury(II) Compounds

As GLC analysis usually requires that an analyte be simultaneously thermally stable and possess volatility, GLC analyses of mercury compounds have involved primarily volatile organomercurials possessing a variety of alkyl, substituted alkyl, or aryl groups.

Isolated reports of inorganic mercury(II) analysis by GLC do exist in the literature, however, and have involved idealized conditions without analytical application. Tadmor (*3*) described the GLC separation of a number of metal halides, including mercury(II) chloride. The solid support was Sil-O-Cel brick, and stationary phases included *n*-butanol, *n*-decane, glycol, aluminum bromide, and bismuth chloride.

In order to achieve the level of GLC detectability of organomercurials encountered in analytical application, it is usually necessary to utilize a gas chromatograph equipped with an electron-capture detector and have the organomercurial in the form of the unsymmetrical halide (bromide, chloride, or iodide) to which electron-capture detectors are especially sensitive. Electron-capture detectors enjoying widest use in mercury analysis are those equipped with tritium (^{3}H) or radioactive nickel (^{63}Ni) foils, the tritium foils having wider use. While the tritium foil is more succeptible to contamination (*4*) by column bleed, thermal decomposition of the organomercurial, and sample impurities it permits analysis at much lower temperatures and with somewhat better sensitivity than the radioactive nickel foil. Other and equally sensitive detection means have been reported and include a mass spectrometer interfaced with a gas chromatograph (*5*), flameless atomic absorption after chemical modification of the column effluent (*6,7*) and emission spectrometry in a helium plasma (*8*). When employing electron-capture detection, diorganomercurials are necessarily converted (*9,10*) to the unsymmetrical monoalkyl- or monoarylmercury(II), dialkyl- and diarylmercury compounds being undetected by this

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means. Conversion is usually to a halide, although Tatton and Wagstaffe (*4*) used the electron-capture detector with organomercurials in the form of dithizonates. The alternative types of detection cited above permit measurements of both monoorganomercurials and the diorganomercury compounds.

A problem frequently encountered in GLC analysis is unambiguous identification of a chromatogram peak as being that of an analyte of interest. Usually one evaluates the chromatographic behavior of an analytical species on a variety of column packings and under varying conditions of column temperature, flow rate, etc., synthetic pure samples being used if available. Comparisons are then made with a suspected component of the analytical sample. In the case of organomercurials of the type RHgX, an alternative approach is that of Nishi and Harimoto (*11*), whereby prior to GLC manipulation samples are treated with solutions of organic thio compounds, a metal sulfide or thiosulfate, or metal powder. Loss of a chromatographic peak on treatment of a sample in this fashion suggests a monomercury compound. A second approach employed by these investigators is a precolumn arrangement in the gas chromatograph. An attractive feature of those methods cited above, i.e., mass-spectrometric interfacing, flameless atomic absorption spectrometry, and emission spectrometry, as alternative detectors to electron-capture obviously is the direct unambiguous identification of a column effluent component as an organomercurial.

To date, column packings which have furnished satisfactory analytical data in the analysis of monoorganomercury compounds include, as stationary phases, the Carbowax series in the higher molecular weight range (*10*), the polyesters diethylene glycol succinate (*12,13*) butane-1,4-diol succinate (*13,14*), diethanolamine succinate (*15*), or OV-17/QF-1 (*8*). Stationary phases employed in the analysis of dialkyl- and diarylmercury include Chromosorb 101 (*8*) and DC-200/QF-1 (*6*). An excellent phase for arylmercury is claimed to be poly(ethylene glycol succinate) in light (1%) loading (*4*).

In general, among a homologous series of unsymmetrical or symmetrical alkyl mercurials, the larger the alkyl group, the longer the GLC retention time or retention volume (*6,10*). Unsymmetrical arylmercury(II) compounds generally elute much later than the alkyl analogs for a given packing (*10*).

A perplexing observation first noted by Westöö (*10*) is the relative lack of effect of the X group in monoalkyl- (*10*) or monoarylmercury (*18*) compounds of the type RHgX on retention time, regardless of packing employed and where a wide variation in steric bulk and polarity of the X group exists. That the various organomercurials may undergo on-column exchange of the X groups was demonstrated by Johansson and Ryhage (*5*), using GLC-mass spectrometry. Mass spectral data for the molecular ions and fragments showed a mixture of methylmercuric iodide and chloride when pure samples of either halide were employed.

Dressman (*18*) has recently reported that, in the GLC analysis of a series of phenylmercury (II) compounds where X in phenyl-HgX varies from halide to carboxylate, diphenylmercury and phenylmercuric chloride emerge solely and in relative amounts varying with the nature of X. These observations were ascribed in some detail to the combined effects of sample contamination by chloride ion and thermally induced homolytic scission in the chromatograph of carbon–mercury and X–mercury bonds, followed by coupling processes involving the radicals produced.

In view of the body of data in the organometallic literature of mercury relating to symmetrization reactions (*19*) of arylmercury compounds as well as the well-studied thermal degradation mechanisms (*20*) of organomercurials, one can assume that the rearrangements do not occur in the solutions of these samples and do indeed proceed in the chromatograph. However, the products one might expect from a simple degradation sequence (*20*) occurring thermally are not fully explained by the chromatographic results (*18*). On-column exchange of X groups with the aryl mercurials, similar to that seen with alkylmercury (*5*) would be an alternative

explanation for the persistent appearance of the chloride.

The upshot of these observations is that caution should be employed when assembling an assay procedure for analysis of phenylmercury or other arylmercury(II) compounds, it being advisable to convert the aryl mercurial to arylmercuric chloride immediately before rapid GLC analysis.

The choice of packing support appears to be of considerable importance, it being desirable to have the support as inert as possible. Divalent mercury compounds of the type RHgX possess linear geometry and a corresponding coordination number of two. Expansion of the metal coordination shell to accommodate other ligands can occur; where the ligand(s) donation arises from packing support (Si-OH groups), adsorption intervenes, though the interaction is not as strong as in the case of nitrogen or sulfur donor groups (*16*). Little problem arises with packings using coordinatively saturated metal compounds, the octahedral tris(trifluoroacetylacetonato)-chromium (III) complex undergoing GLC analysis without difficulty *(1,2)*. In this connection, it has been reported that certain packings require periodic "dressing" in routine use *(17)*.

This problem may be minimized or eliminated in several ways. More efficient or thorough coating of the support by the stationary phase could be carried out by fluidizing, where final work-up of a packing involves the use of an apparatus employing fluid bed principles. Such an apparatus is commercially available from Applied Science Co., State College, Pa., and has been employed by this author with good results. A better approach is the use of packings in which the stationary phase is chemically bonded to the support, essentially eliminating active binding sites as well as minimizing phase bleed problems, resulting in reduced sample hold-up and detector contamination. Durapak Carbowax 400 (low K') bonded to Porasil F, commercially available from Waters Associates, Inc., Framingham, Mass., has been used successfully in this laboratory. No periodic treatment of the packing in routine use is necessary.

GLC Analysis of Alkylmercury(II) Compounds in Various Media

With increased awareness of the toxicological aspects of certain organomercurials notably the lower alkyl compounds *(21–23)*, a good deal of attention has centered specifically on GLC techniques for the analysis of the neurotoxic methylmercury(II).

Analytical applications of GLC in the above regard have embraced a wide variety of foodstuffs as well as environmental media, each matrix posing its own set of analytical problems.

In a series of papers (*10,15,24,*), Westöö et al. have described GLC techniques for the evaluation of monomethylmercury(II) as well as dimethylmercury(II) in fish, eggs, meat, liver, kidney, algae bile, blood, moss, sludge, and sediments. Later reports by other workers (*8,14,17,25*) have involved variations of Westöö's technique.

In the Westöö procedure, after successive published refinements of methodology, the initial step in the analysis of monomethylmercury(II) involves liberation of the monoalkyl mercurial from the matrix to which it is bound. Binding involves the methyl thio group or sulfhydryl and/or amino groups of proteins, peptides, and amino acids in cells and at cell membranes (*26*). Cleavage of the methylmercury from various binding groups is achieved with hydrochloric acid in concert with other reagents to optimize cleavage. Fish, eggs, meat, bile, and algae are homogenized and treated with concentrated HCl to yield a final mixture concentration ca. 2*N* in HCl. Sodium chloride is added, and extraction of the liberated methylmercury is carried out with benzene. The benzene extract is then reextracted with a cysteine salt solution into an aqueous phase as the cysteine complex. After treatment with HCl, the liberated methylmercury is again partitioned into benzene. In a procedural variation for fish, egg white, kidney, blood, meat, bile, algae sediment, moss, and sludge, mercuric chloride solution is added to the liquid medium or homogenate prior to the initial extraction with benzene, the inorganic mercury serving

to assist in liberation of the methyl mercury via binding site competition. For analysis of methylmercury in liver, the homogenate is treated with molybdic acid in lieu of mercuric chloride. Westöö reports that when acidified liver suspensions are kept for extended periods of time in the presence of added mercuric chloride and worked up, the amount of methylmercury increases relative to shorter-term analysis.

Other authors (*25,27*) have used hydrobromic acid in place of HCl–NaCl at this stage. In place of mercuric chloride or molybdic acid, copper (II) ion has been employed (*25*). Toluene may be used in place of benzene (*25,27*) as organic extracting agent. For analysis of cereal grain products, a mixture of benzene and formic acid (10:1 v/v) was found to yield better partitioning data than benzene alone (*14*).

While conventional homogenizing of media appears to work in most cases, Newsome (*14*) has reported better results by use of a twofold homogenizing step and by interposing a preliminary filtration of the first homogenate over glass wool. In the case of cereal grains, futhermore, he finds that an additional cleanup entailing passage of the filtrate (from glass wool separation) through a silicic acid column constructed in benzene yields good results.

The organic extract containing the liberated methylmercury is relatively dirty, chromatographically speaking, and further cleanup of the sample extracts is usually necessary. In the Westöö procedure, a second extraction of the organic layer with aqueous solutions of cysteine salts in varying amount is carried out, followed by acidification of the methylmercury- containing cysteine solution with HCl and repartitioning of the liberated methylmercury-containing cysteine solution with HCl and repartitioning of the liberated (*13*). Further variations of this step in the assay procedure are the use of aqueous or aqueous ethanolic solutions of thiosulfate in place of the organic thio compounds (*25,27*). Addition of iodide ion is followed by extraction of methylmercury as the iodide.

Tatton and Wagstaffe (*4*) evaluated the alkylmercury fungicides methyl-, ethyl-, methoxyethyl-, and ethoxyethylmercury as residues from apples, potatoes, and tomatoes using thin-layer and gas-chromatographic techniques. Since the alkoxy-substituted alkylmercury compounds are labile to acid treatment, as would be encountered in the Westöö procedure, an alternative extraction-isolation technique was employed. Treatment of the appropriate samples with a slightly alkaline solution of cysteine in isopropanol is followed by washing of the extract with diethyl ether or toluene and treating the organomercurials extracted with an ether solution of dithizone. As residues are concentrated on the skin of the foodstuff in the case of apples and potatoes, the samples are peeled and the peels chopped. With tomatoes the entire sample is macerated.

The final organic extracts in the various procedures are dehydrated over a drying agent, usually anhydrous sodium sulfate, prior to GLC analysis. Some caution should be employed in choice of drying agent, spurious peaks from contaminating compounds in the drying agent having been observed in some cases (L. Goldwater, private communication). Prior testing of the drying agent by addition of a sample of desiccant to pure benzene followed by GLC analysis should be carried out with each new container of desiccant. Contaminated containers should be replaced, or batch extraction of the agent with pure benzene carried out until clean chromatograms are obtained. This author has routinely employed anhydrous magnesium sulfate as drying agent (Baker and Adamson) with no contaminants ever being observed.

Instrumental parameters employed for GLC analysis of the dried organic extracts are those described above.

Where dimethylmercury analysis is desired in the presence of monomethylmercury, one may do a differential analysis involving extraction of the medium prior to acidification and addition of mercuric chloride (*9,10*), since halogen acids or mercuric chloride convert dialkylmercury to the unsymmetrical mercurial. Dialkylmercury compounds such as dimethylmercury are not bound to the matrix and are easily extracted. Treatment of the

organic layer with mercuric chloride yields monomethylmercury, which is then subjected to GLC analysis. Any dimethylmercury determined in this fashion is then subtracted from the amount determined in the direct acidification method.

Arylmercury(II) Analysis by Gas-Liquid Chromatography in Various Media

In contrast to the fairly extensive literature for bioanalysis of methylmercury, little is available in the way of assay procedures for the corresponding aryl analogs. This is due in part to the recognized lower level of toxicity of aryl mercurials (*26*) which are readily degraded by a variety of organisms. Matsumura et al. (*28*) have described an assay procedure for analysis of phenylmercury by combined paper, thin-layer and gas-liquid chromatographic techniques. The analytical procedure was developed for evaluation of the fate of phenylmercuric acetate in the presence of various sediment bacteria. Supernatants obtained from centrifuging the microbial media are acidifed with sulfuric acid, sodium chloride added, extracted with benzene, the extracts filtered through a Florisil column, and the eluate analyzed by GLC. The arylmercury fungicides, phenyl and tolylmercury, appearing as residues on various foodstuffs have been measured by GLC techniques by procedures identical to those for alkylmercury compounds (*4*) as described in the previous section.

Relative Merits and Limitations of Gas-Liquid Chromatography in the Analysis of Mercury(II) Compounds.

From the standpoint of environmental health and pathology, major interest resides with the lower alkyl mercurials, particularly methylmercury. On a relative toxicity scale, methylmercury is much more toxic than inorganic mercury(II), and hence the assessment of methylmercury or other lower alkylmercury in a medium of importance to environmental health poses more analytical urgency. That GLC is becoming accepted as a routine technique for the evaluation of organomercurial content is apparent.

The GLC technique as presently employed permits measurements of only organomercurials in various media, it being necessary to resort to neutron activation analysis or flameless atomic absorption spectrometry for levels of total mercury. As pointed out above, knowledge of the organomercurial content of a sample is satisfactory in most instances, but in cases where one chooses to do studies dealing with the metabolic fate or other transformations of mercurials it is highly desirable to ascertain levels of inorganic and organic mercury and to do so without recourse to widely disparate techniques. In a recent report by Magos *(29)*, techniques are described for evaluation of the inorganic and organic mercury content of a given sample. This procedure has the marked advantage, highly desirable in analytical chemistry, that essentially identical manipulation and instrumentation are employed for evaluation of both forms; although the specific nature of the organomercurial cannot be determined, in practice any organomercury is assumed to be in the form of methylmercury.

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